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Synthesis, Characterization and Molecular Docking Studies of Novel Pteridine Derivatives

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***Abstract*: Pteridines are a group of heterocyclic compounds which contain nitrogen as a major heteroatom and it was aromatic compounds that are attached to the pyrazine and pyrimidine ring family. Pteridine derivatives have focused on a specific place in the medicinal chemistry field. The Pteridine ring is present in several natural compounds such as Pterin, Hemiptera (erythropoietin), Biopterin, Neopterin, Xanthopterin, iso-xanthopterin, etc.., which include a few heterocyclic compounds like pyrimidine, pyrazine rings. Some vitamins like folic acid and riboflavin and drugs like methotrexate and triamterene. A pteridine is formed by a condensation process. Pteridine is frequently produced using costly and dangerous chemicals. In order to reduce the cost and consumption of hazardous chemicals, novel pteridine compounds were made by mixing a catalyst with a solvent, primary amines, and phenols. Currently, the primary issue limiting a medicine's ability to effectively treat a disease includes development of resistance. In this article, we will utilise docking studies to provide a thorough explanation of the synthesis of pteridine derivatives and their antioxidant capabilities.**

***Keywords*: Pteridine, Isay reaction, Anti-oxidant, DPPH, Molecular docking**.

INTRODUCTION

Since most components with biological activity are produced from heterocyclic structures, the synthesis of heterocyclic systems is of continuous interest in the study of organic chemistry. A broad range of pharmacological activities, including anticancer, anti-inflammatory, antioxidant, anti-leishmanial activity, anti-diuretic, antimicrobial, antiparasitic, and some of the derivatives supposed to act as a myocardial infarction, have been observed for pteridine derivatives, an interesting class of privileged heterocycles with promising biological and therapeutic activity [1]. The production of folic acid employs substituted pteridine derivatives as a precursor. Examples of substituted pteridine compounds include folic acid and riboflavin. Similar to this, the pteridine ring system is similarly a favoured hetero system and may be found in many compounds, both natural and artificial, that have important pharmacological effects. One of the strongest techniques in rational drug design for creating novel structurally varied pharmaceuticals (with fused heterocycles) is the annulation or inclusion of two or more heterocyclic systems, as structural variety is obviously associated with the compound's potentiality endowed has therapeutic qualities. Pteridine's annulation with other hetero systems including pyrazine and pyrimidine, as well as its existence in natural products, suggest a variety of biological characteristics. We have designed and synthesized pteridine derivatives, based on two privileged biodynamic heterocyclic scaffolds, energized by the promising biological activities of the structurally diverse heterocycles with fused

heterocyclic systems and our ongoing research program on the synthesis of therapeutically interesting heterocycles. By using DPPH radical scavenging tests, the produced pteridine derivatives were evaluated for their antioxidant properties. The antioxidant activity of the produced substances has also been assessed. We have looked at pteridine derivatives having hydroxymethyl, carboxylic, chloroethyl, and amino groups on the ring. Most of them are recognized as being natural substances [2]. They have demonstrated excellent anti-oxidant and radical scavenging abilities.

MATERIALS:

Docking steps

Protein selection: (NCBI) National Center for Biotechnology Information, Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) [3].

Ligand optimization: to predict a position and orientation of a small molecule (ligand) to a larger receptor molecule (macromolecule) with minimum binding energy.

Autodock: perform computational molecular docking of small molecules to proteins, DNA, RNA and other important macromolecules.

Pymol:3D visualization of proteins.

Molgrow: detects and visualizes the interactions between ligand & protein and helps to find number of hydrogen bonds.

Websites used in docking

SwissADME: used to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of ligands [4].

Protein-ligand interaction profiler: detects and visualizes the interactions between ligand & protein and helps to find a number of hydrogen bonds [5].

Hyperchem: 3D visualization of ligand

RSCB PDB: protein downloading (homology modeling done) [6].

CASTp 3.0: (Computed Atlas of Surface Topography of Proteins) to study surface features and functional regions of proteins (active sites of protein).

Open Babel: change file format.

SPDBV: (Swiss-PdbViewer) used to perform homology modelling of a protein**.**

SAVES 6.0: Verification of protein structures: patterns of nonbonded automatic interactions.

METHODOLOGY:

METHOD

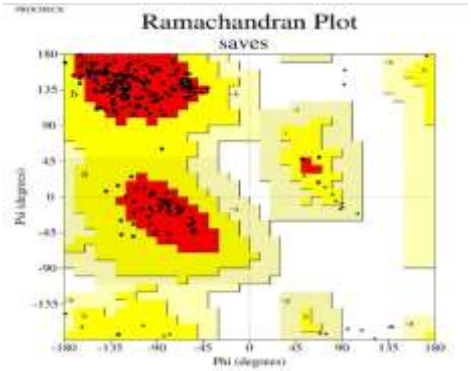
Homology modelling

The target sequence of 7RG7 was accrued from UniprotKB protein knowledge base and compelled by using NCBI PSI-BLAST to identify the template sequence. It was existing as a good source to predict structure-based pharmacophore analysis. Further, the three-dimensional protein structures were built by using Swiss PDB Viewer (SPDBV) and the protein structure by Modeller 9.12.

Ramachandran plot of protein model

SAVES OF 7RG7

VERIFY 3D- 80 % of the residues have averaged 3D-1D score >=0.2; PASS **ERRAT**- Overall quality factor is 86.166

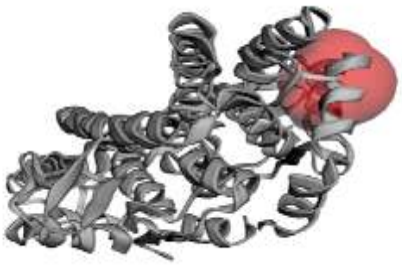


Plot statistics

Residues in most favoured regions [A,B,L]	210	92.5%
Residues in additional allowed regions [a,b,l,p]	17	7.5%
Residues in generously allowed regions [~a,~b,~l,~p]	0	0.0%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	227	100.0%
Number of end-residues (excl. Gly and Pro)	4	
Number of glycine residues (shown as triangles)	26	
Number of proline residues	7	
Total number of residues	264	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

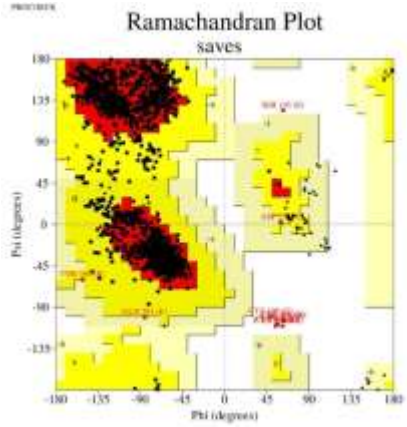
- ❑ Active site of 7RG7 protein predicted by CASTp server



ASP40, PHE42, VAL43, PHE45, LYS46 and LYS49.

SAVES OF 4L8w

VERIFY 3D- 80 % of the residues have averaged 3D-1D score >=0.2; PASS **ERRAT**- Overall quality factor is 86.142

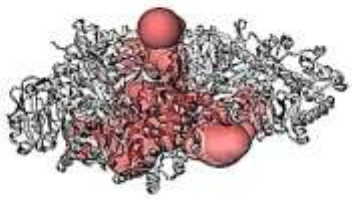


Plot statistics

Residues in most favoured regions [A,B,L]	1388	91.1%
Residues in additional allowed regions [a,b,l,p]	126	8.3%
Residues in generously allowed regions [~a,~b,~l,~p]	3	0.2%
Residues in disallowed regions	7	0.5%
Number of non-glycine and non-proline residues	1524	100.0%
Number of end-residues (excl. Gly and Pro)	12	
Number of glycine residues (shown as triangles)	96	
Number of proline residues	90	
Total number of residues	1722	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

- ❑ Active site of 4L8w protein predicted by CASTp server



ASP46, PHE48, VAL47, PHE41, LYS45, SER178, SER179 and LEU180.

Ligand preparation:

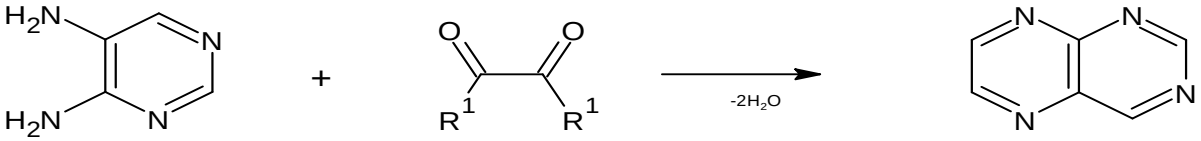
The novel pteridine analogues are Pteridine-2,4-diamine, (2,4-diaminopteridin-6-yl)methanol, 2,4-Diamino-6-(hydroxymethyl)pteridine hydrochloride, 6-((Bis(2-chloroethyl)amino)methyl)-2,4-pteridinediamine, 2,4-Diamino-6-chloromethylpteridine,6-phenylpteridine-2,4,7-triamine, 6-acetyl-1,3-dimethylpteridine-2,4(1*H*,3*H*)-dione
2-amino-6,7-di(pyridin-2-yl)pteridin-4(3*H*)-one, 7-{[(naphthalen-2-yl)amino]methyl}pteridine-2,4-diamine, 1-(2-amino-4-oxo-4,4a-dihydropteridin-7-yl)-4-(hydroxyamino)butane-1,3-dione, 2-{[(quinolin-2-yl)methyl]sulfanyl}pteridin-4-ol
, 6,7-dimethyl-2-{[(quinolin-2-yl)methyl]sulfanyl}pteridin-4-ol, 2-{[(3,5-dimethyl-1,2-oxazol-4-yl)methyl]sulfanyl}-6,7-dimethylpteridin-4-ol, 2-[(2-imino-6,7-dimethyl-1,2-dihydropteridin-4-yl)amino]ethan-1-ol, 4-{[(2,4-diaminopteridin-7 yl)methyl]amino}benzene-1-sulfonamide, 6methyl-2-phenyl 4 amino pteridine, were retrieved from PubChem database (http://pubchem.ncbi.nlm.nih.gov/search/search.cgi). The ligand optimization was performed by Hyperchem Professional 7.0.

Molecular Docking:

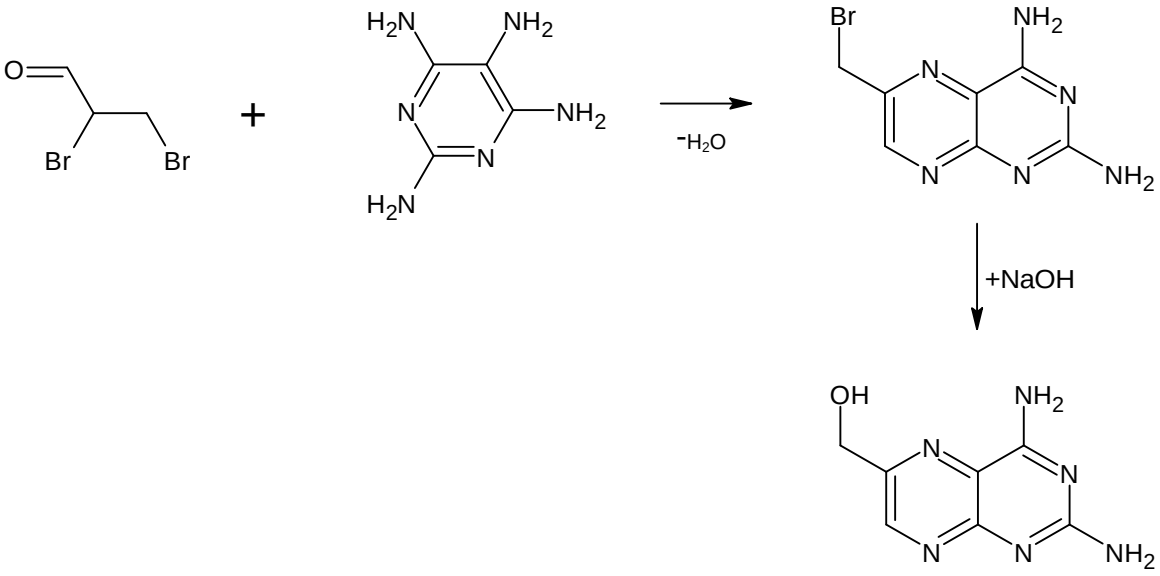
The docking evaluation of ligands (pteridine derivatives) were docked with 7RG7 protein using Auto dock tools. Molecular docking studies were carried out using Auto dock 4.2 and Auto Dock Tools 1.5.4 from the Scripps Research Institute, http://www.scripps.edu/mb/olson/doc/autodock. [7]
The Lamarckian genetic algorithm was used for ligand conformational searching. The local search algorithm, which builds a population of individuals (genes), each being a different random conformation of the docked molecule. The grid was generated around the active site at 80 × 80 × 80 to calculate molecular simulation using AMBER tools, showed auto grid of active site residues around the complex structure. There were 150 populations with a mutation rate of 0.02, crossover rate of 0.8 and default grid spacing 0.375Å were used as parameters settings for docking. Consequently, these simulations were performed using up to 2.5 million energy evaluations with a maximum of 27,000 generations and each simulation was performed by 10 times that yielded 10 docked conformations. At last, the lowest energy conformations were regarded as the binding conformations between ligands and the protein.

General method of preparation:

Synthesis involves the condensation of 1,2 dicarbonyl compound with diamino pyrimidine with suitable take up position 6 and 7 with substituted of R_4 and R_5 in round bottom flask at 100-160°C in presence of glacial acetic acid.



Synthesis of 2,4-diamino-6-hydroxymethyl pteridine



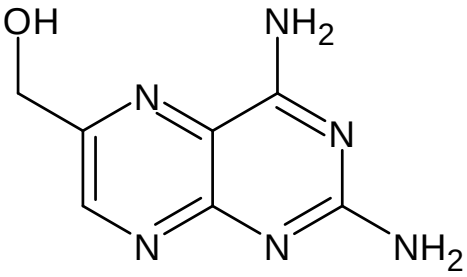
Method of collection of data:

Practical data will be obtained from laboratory-based studies

- Synthesis
- Testing purity by TLC
- Characterization of compounds by IR, NMR and Mass spectroscopy.
- Biological activity confirmed by docking studies.

Synthesis of Pteridine derivatives

Structure: 2,4-diamino-6-hydroxymethyl-pteridine



Mobile phase preparation: n-Hexane: Ethyl acetate (7:3)

RESULTS AND DISCUSSION

Table 1: Binding energy of docked compounds

Table 2: Solubility data of the compounds

Ligand	Binding free energy (Kcal/mol)	RMSD (A)	Inhibition constant KI (µm)	No. of Hydrogen Bonds
1A	-5.09	129.972	186.53	2
1B	-4.57	130.941	449.47	3
1C	-5.14	129.175	169.50	0
2A	-4.38	132.203	615.48	1
2B	-4.58	133.305	437.31	2
2C	-4.79	137.802	305.70	0
3A	-4.59	129.435	433.74	0
3B	-4.56	129.421	457.21	0
3C	-5.40	131.542	109.42	1
3D	-5.45	130.844	101.09	0
4A	-5.12	128.968	175.37	0
4B	-4.63	137.776	401.98	0
4C	-4.56	136.517	451.72	1
Standard	-4.69	128.865	367.52	4

Solvents	Synthesized compound
Methanol	Soluble
Ethanol	Soluble
n-Hexane	Insoluble
Ethyl acetate	Completely soluble
Toluene	Insoluble
Petroleum ether	Insoluble
Water	Insoluble

Table 3: Physical properties of the synthesized compound

Physical properties	Compound 1A
Molecular Formula	$C_7H_8N_6O$
Molecular Weight	192.18
Appearance	Yellow colour
Melting Point	295-300°C
Rf Value	0.73
Yield	80%

Interpretation of the lead compound

IR spectroscopy is used to establish whether a given sample of an organic substance is identical to another or not. This is because a large number of absorption bands is observed in the IR spectra of organic molecules and the probability that any two compounds will produce identical spectra is almost zero. So if two compounds have identical spectra then both of them must be sample of the same substances.

Infrared analysis of the standard showed identical "fingerprint regions". The following frequencies (wavenumbers) were shown to be characteristic of both molecules: 3300-3500 (amines, N-H stretch), 1690-1760 (ketones, C = O stretch), 1600 (aromatics, C = C), 1180-1360 (amines, C-N), and 675-870 (aromatic rings, C-H). C-H stretching vibrations usually appear between 3200 and 2800cm^{-1} and carbonyl(C=O) stretching vibrations usually appear between 1800 and 1600cm^{-1}. The region of the infrared spectrum from 1200 to 700 cm^{-1} is called the fingerprint region. This region is notable for the large number of infrared bands that are found there. Many different vibrations, including C-O, C-C, and C-N single bond stretches, C-H bending vibrations, and some bands due to benzene rings are found in this region.

Figure 1 IR interpretation of synthesized compound

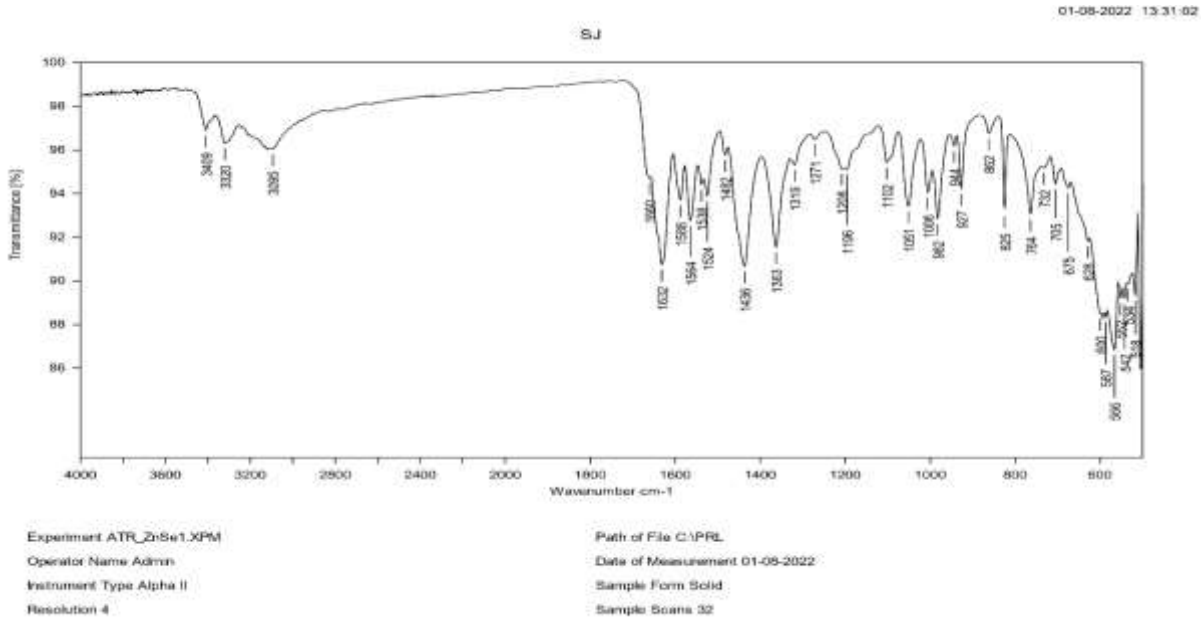
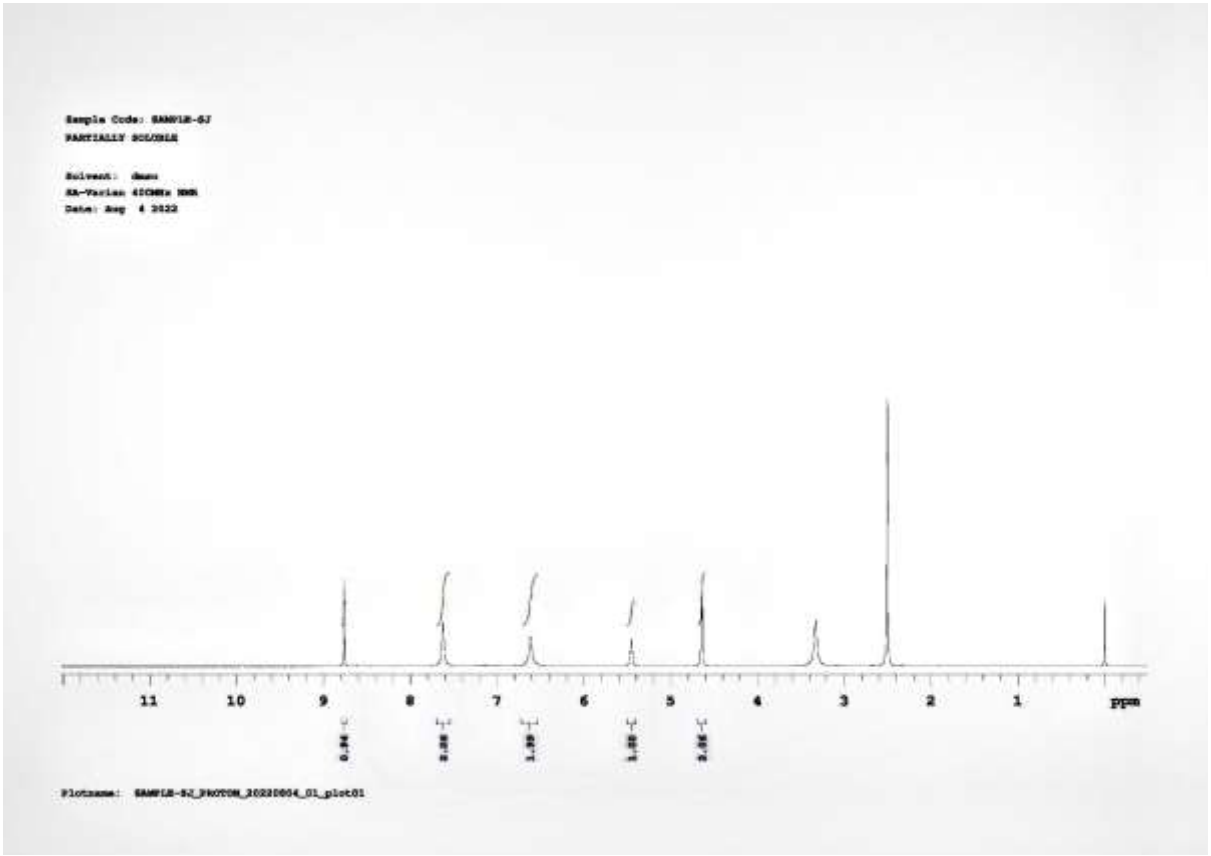
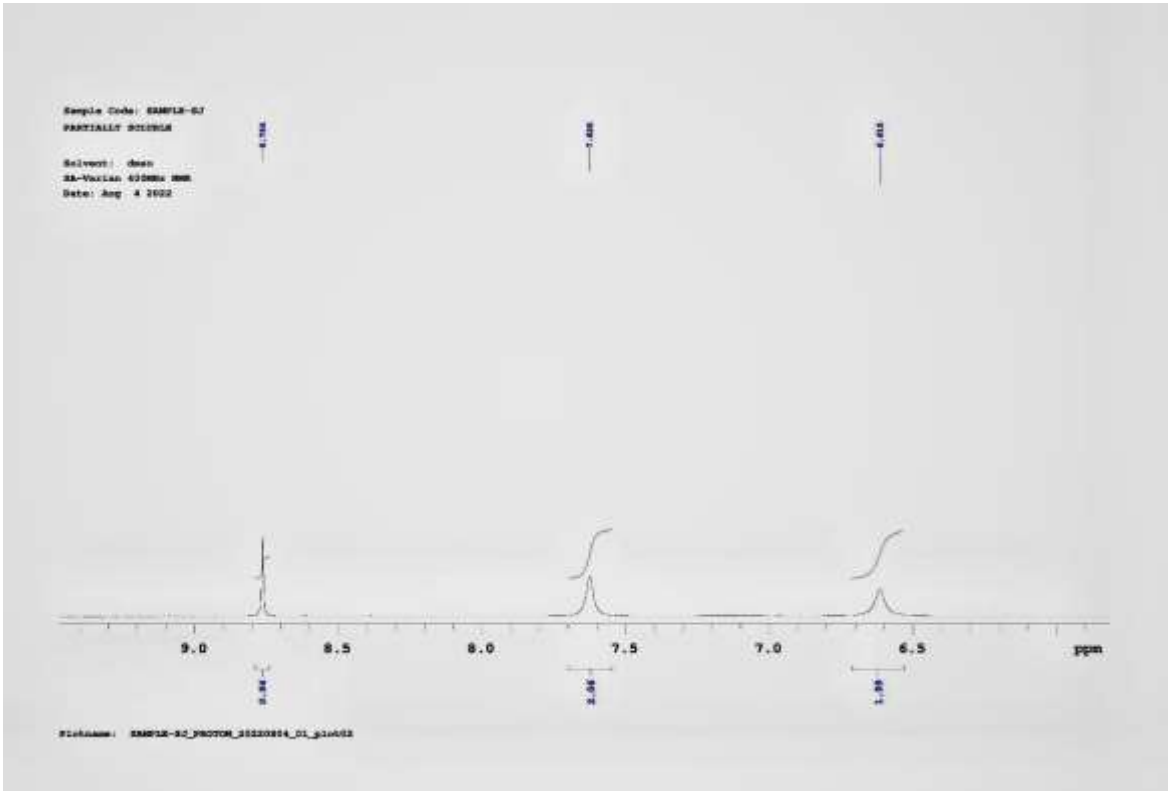


Figure 2 NMR of synthesized compound




9.0
8.5
8.0
7.5
7.0
6.5
ppm

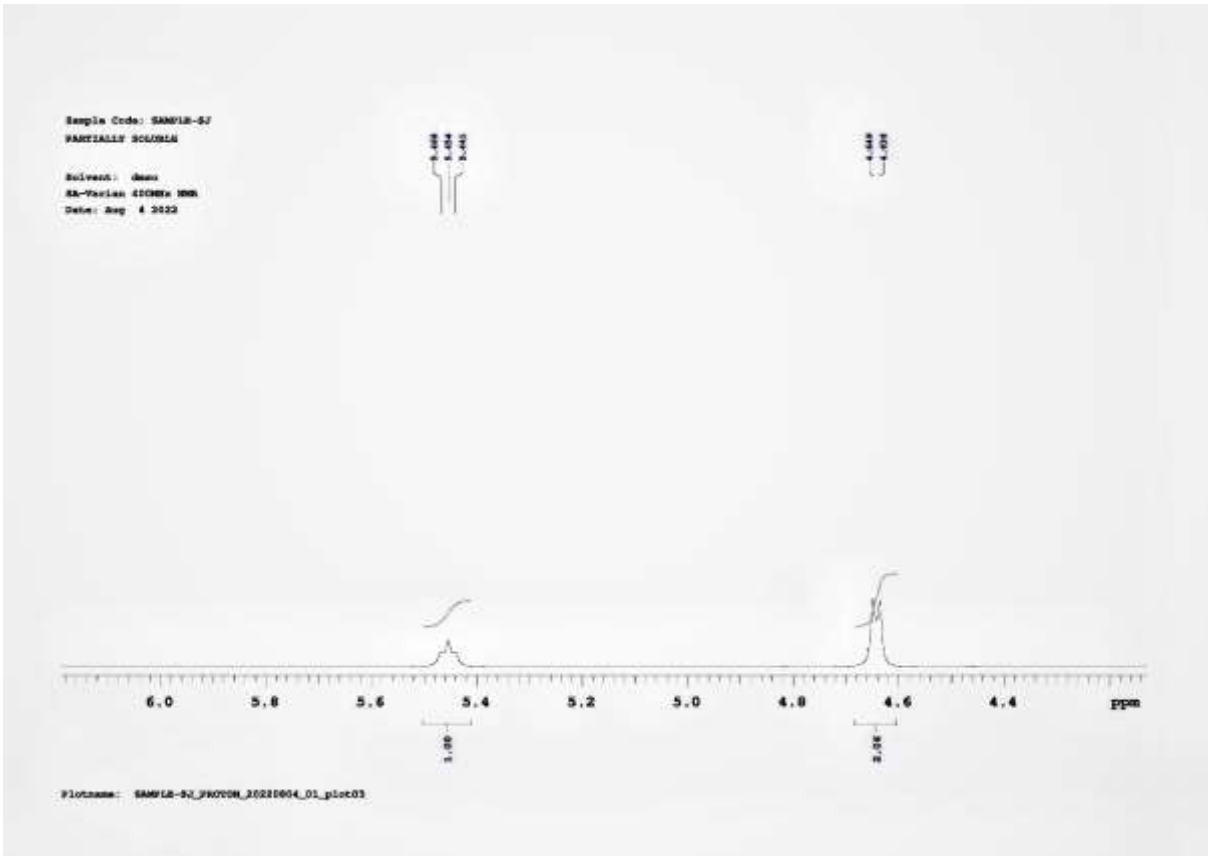

6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
ppm

Table 4 ADMET prediction of Ligand molecules

Ligand	Carcinogenicity	Oral acute toxicity	LogS (water solubility)
1A	0.5163	0.6142	-3.4641
1B	0.5734	0.6476	-3.2269
1C	0.5533	0.6503	-3.6295
2A	0.6032	0.7048	-3.3377
2B	0.5528	0.6831	-3.6648
2C	0.5858	0.6965	-3.8107
3A	0.6032	0.7048	-3.3377
3B	0.5528	0.6831	-3.6648
3C	0.5858	0.6965	-3.8107
4A	0.5986	0.6011	-3.8770
4B	0.5533	0.6503	-3.6295
4C	0.5163	0.6142	-3.4641

CONCLUSION

A significant element in the creation of drugs using a structure-based methodology is the protein-ligand interaction. In the latest research, we have taken the receptor protein and targeted the oxidants-using medications. Following data analysis, compound 1 was found to have the lowest binding energy and lowest inhibition constant, making it the best molecule for bonding to the 7RG7 protein and a potential lead molecule for antioxidants. The energy value observed after the medicine methotrexate docked with the receptor 7RG7 was (-4.69). The energy value found was (-5.09) when the developed medicines (compound 1A) were docked against the same receptor. This leads us to believe that certain created medications are preferable to those that are available commercially. Then, after synthesizing and evaluating these compounds that had more activity than the available commercial medication, Future research might include testing these synthetic medications' pharmacokinetic characteristics in a wet lab and moving forward with clinical trial research. The remaining compounds which were docking have the least binding activity compared to the above compound by the data obtained by autodocking studies.

Acknowledgement
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